

RETENTION OF WATER BY PLANT CELL WALLS AND IMPLICATIONS FOR DROUGHT RESISTANCE

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Summary

The water-retaining properties of cell wall materials from the roots of two monocotyledons (*Ehrlharta calycina* Sm. and *Triticum vulgare* Vill. cv. Gabo) and two dicotyledons (*Salicornia australis* Banks & Soland and *Vicia faba* L.) have been studied.

Differences in the sorption-desorption isotherms found for the various wall materials suggest that an important factor enabling a drought-resistant species to endure prolonged water stress is the relatively greater tenacity with which its cell wall water is held in comparison with that of mesophytes.

I. INTRODUCTION

A considerable proportion of "apparent free space" (AFS) or "outer space" is present in plant tissue (Kramer 1957). For example the AFS of wheat roots has been estimated to be as high as 33.5% of the root volume (Butler 1953). Estimates of the amount of AFS vary with the method of determination, but values for the free space of roots obtained by various investigators range from about 12 to 35%.

Many workers, including Butler (1953), Levitt (1957), Dainty and Hope (1959), Laties (1959), Kramer and Kozlowski (1960), hold the view that the AFS resides entirely or largely in the cell wall. More recently electron-microscopic and other studies of the AFS of root cells of pine seedlings by Salyaev (1963, 1964) in Russia have provided convincing evidence that the AFS is in fact confined to the cell wall, and that a mass flow of water along the wall does actually occur. These findings are reasonable in view of the numerous interfibrillar spaces present in plant cell walls. The cell wall is considered as a micro- and macropore system (Dainty and Hope 1959) with the micropores (≤ 100 Å in diam.) leading into the macropores (≥ 100 Å in diam.). As a result of the numerous electron-microscopic studies on plant cell walls the presence in cellulose of individual microfibrils can now be considered as well established (Odintsov 1957).

Recent electron-microscopic work by Gaff, Chambers, and Markus (1964) have also demonstrated the presence of a "facile transport system" in the cell wall in which channels intercommunicate freely. The cell wall is generally considered a direct flow extension of the xylem system (Scholander *et al.* 1965). According to Raney and Vaadia (1965) the macropores of the root system are probably in the cell walls, and under hydrodynamic conditions of transpiration most of the water flow occurs in these larger pores.

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In studies of the water relations of plants, the water in the cell wall has received increasing attention in recent years. It has been suggested that the considerable amount of water present in cell walls (actually the AFS) could act as a buffering system during periods of water stress since the resistance to movement is relatively lower along the wall than through the wall (Carr and Gaff 1961; Gaff and Carr 1961; see also May and Milthorpe 1962). However, no explanation has so far been put forward as to why this so-called buffering capacity is not as effective in drought-sensitive species as in drought-tolerant ones during drought periods.

In this paper the sorption-desorption isotherms for root cell wall materials of different species are compared. The implications of the relative amount of water sorbed, as well as the accompanying hysteresis, are discussed in relation to drought resistance. As pointed out by Slatyer (1965), an understanding of the relative mobility and physiological activity of various cell water fractions is a desirable prerequisite to further progress in studies of cell water relations.

II. MATERIALS AND METHODS

Cell walls from which the amorphous constituents have been extracted are sometimes referred to as pure cell walls. These "purified" wall materials are in fact simply cellulose skeletons and unrepresentative of the natural condition as found in the plant. For this reason, cell wall materials used in this study were prepared by removing only the cytoplasm from the cells, leaving the amorphous matrix of the wall intact. It was hoped that the use of wall materials prepared in this manner would provide a more meaningful comparison between the several species studied.

Two dicotyledons (*Salicornia australis* Banks & Soland and *Vicia faba* L.) and two monocotyledons (*Ehrharta calycina* Sm. and *Triticum vulgare* Vill. cv. Gabo) were chosen for their contrasting botanical differences. Wall materials from the young roots of these plants were prepared using a standard method, so that data obtained could be directly compared.

The freshly harvested roots were washed thoroughly until free of soil particles; they were comminuted in 0.5M sucrose in a MSE Ato-Mix for 15 sec, and the mixture strained through muslin. This process was repeated a further two times. The residue was then washed with 10% NaCl four times (again using the Ato-Mix), each time the excess solution being squeezed out by hand as it would not drain of its own accord. To saturate the material with calcium, it was then washed four times with 20% CaCl₂. Finally, it was washed with distilled water until free of chloride; the absence of chloride was tested by adding AgNO₃ to the washings. The above operations were carried out in a cold-room at 4°C. After air-drying at 20°C the wall material was passed through a 1-mm sieve of an EBC mill (Casella, London).

For ease of handling, particularly in sampling, the wall materials (air dry) were compressed into small cores at a pressure of 500 lb/in². This was achieved by means of a stainless steel mould and a hydraulic press: 30 mg was found to be the minimum amount of material necessary to make suitable cores, both from the point of view of coherence and the time taken to reach equilibrium.

The cores were allowed to sorb water until equilibrium was reached at particular values of water stress. A range of equivalent water suctions was obtained by using

improved versions of pressure plates (Richards and Fireman 1943), pressure membranes (Richards 1941, 1947), and vacuum desiccators containing saturated salt solutions (O'Brien 1948) for the low, middle, and high suction ranges, respectively. The first two techniques involve the application of a constant air pressure to one side of a ceramic plate or cellulose membrane, the other side being in contact with a reservoir of the appropriate solution. This air pressure induces a curvature in the meniscus of each membrane pore equivalent to that produced by a hydrostatic suction applied to the solution in the membrane. Higher equivalent suctions (i.e. lower vapour pressures) are more conveniently obtained by equilibration with the constant vapour pressures obtained over various saturated salt solutions. Sorption of electrolyte solutions was examined by replacing the water in the pressure plates and pressure membranes with solutions of different concentrations. These determinations were confined to the liquid transport range, since the vapour pressure measurements involve a continuous variation in osmotic pressure component of the total water potential as the water content changes, for all but salt-free materials.

This equipment was housed in a constant-temperature room maintained at $20 \pm 1^\circ\text{C}$. Due to the enclosed nature of the equipment thermal fluctuations in the immediate environment of the cores would be much smaller than those in the room itself. In the use of the pressure plates a system of precision air-pressure regulators (Negretti and Zambra, London) in conjunction with mercury manometers and water columns was used to adjust the water potential to any desired value.

Sampling was carried out by removing cores in duplicate from the apparatus and immediately transferring them into stoppered weighing bottles. Water contents were calculated on the basis of the oven-dry weight of the cores at 105°C . After drying, the cores were discarded since heating to 105°C did in fact lower the sorptive capacity of the wall materials when re-wetted. Many fibres including wool and cotton also show an appreciable decrease in sorption after they have been dried at 105°C for 24 hr (Chabert and Diemunsch 1962). However, weight losses between drying under vacuum over P_2O_5 and drying in the oven at 105°C were found to be insignificant. The former method was not used as several days were required for the wall material to attain constant weight.

The growth of *Mucor* spp. and other Phycomycetes on the cores at low suction was prevented by painting a solution of thymol in alcohol on the roof of each Perspex dome of the pressure plates.

Preliminary experiments indicated that the approach to equilibrium (using 30-mg cores) was asymptotic, but in general some 18, 8, and 12 days were found to be satisfactory times for pressure plates, pressure membranes, and desiccators, respectively. The penetration of the water molecules into the internal network of fine capillaries in the cell wall could be expected to be a relatively slow process, proceeding initially with interfibrillar and finally with intrafibrillar swelling.

III. RESULTS AND DISCUSSION

Figure 1 shows the sorption-desorption isotherms obtained for the four species. Water content has been plotted versus water potential expressed as the logarithm of

the applied suction in millibars (Slatyer and Taylor 1960, 1961; Taylor and Slatyer 1961*a*, 1961*b*). In general, the water uptake increases exponentially as the water stress decreases. This may be attributed to the nature of the internal adsorption.

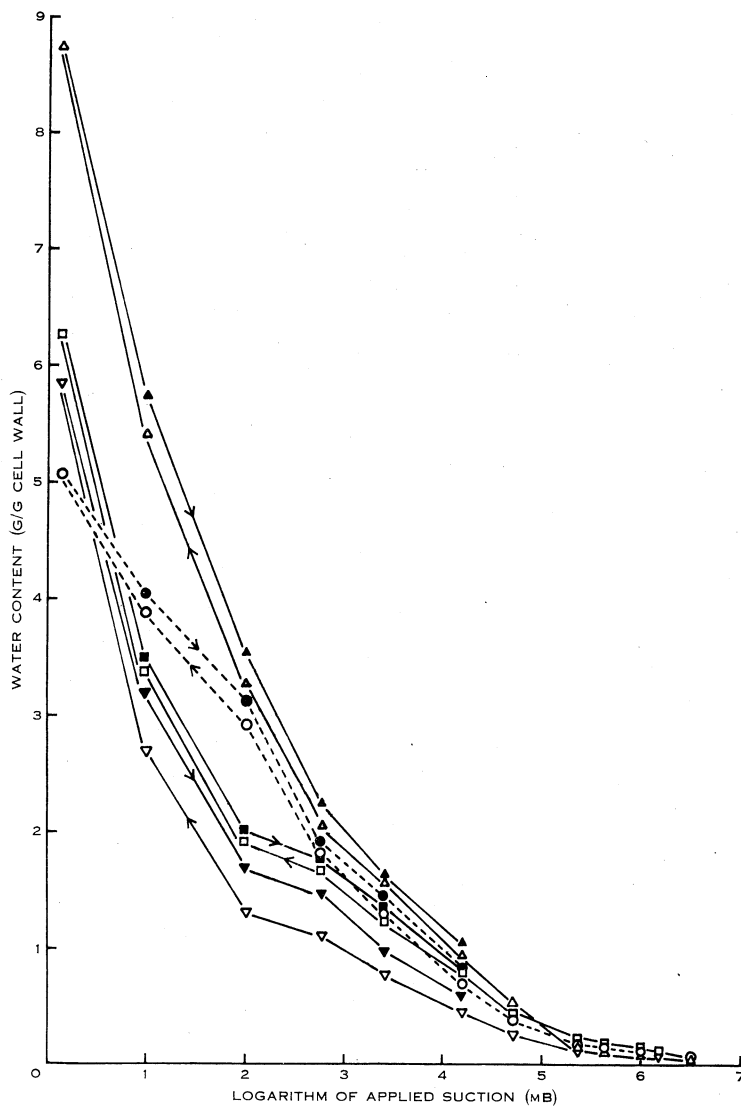


Fig. 1.—Sorption-desorption isotherms at 20°C for the cell wall-water system for the four species. Cell wall preparation saturated with calcium as described in the text. Solid symbols denote desorption, open symbols sorption.
 ▲, △, *T. vulgare*; ●, ○, *S. australis*; ■, □, *V. faba*; ▼, ▽, *E. calycina*.

As swelling of the cell walls proceeds, the opening up of the matrix exposes fresh sites for the further adsorption of water molecules, the amount sorbed depending on the number and position of the polar groups present on the macromolecular chains. However, the large water uptake at low water stress is probably due to capillary

absorption between the wall fragments constituting the core. In the absence of this absorption, the isotherms may well be sigmoid-shaped as has been observed for many cellulosic materials. It is difficult to make an exact distinction between such capillary absorption and purely surface-adsorbed water. However, preliminary studies with fractions of wall materials of various particle sizes indicated that the contribution from such macrocapillary water is insignificant at values of water suction greater than some $10^{1.3}$ mb.

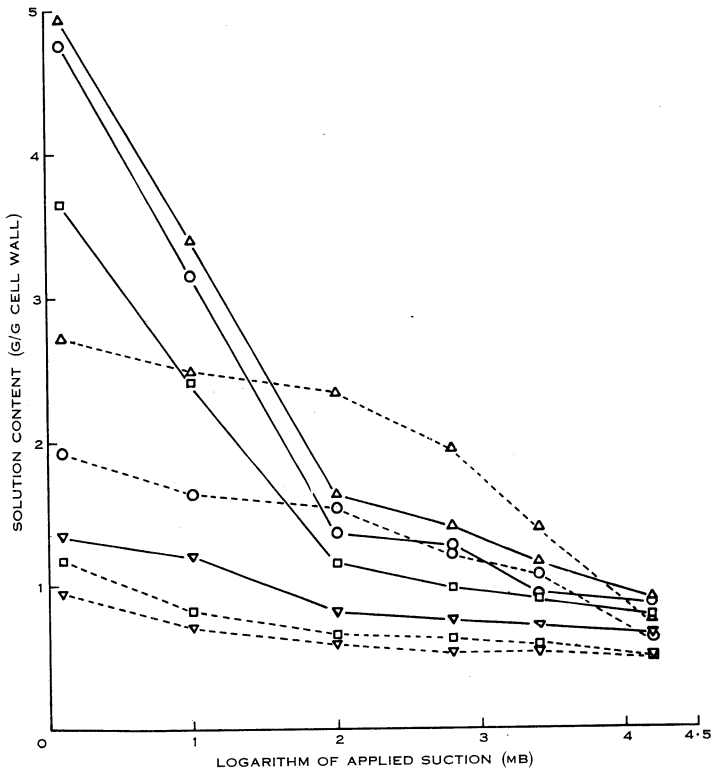


Fig. 2.—Sorption isotherms at 20°C for the cell wall-calcium chloride system for two species at four concentrations of calcium chloride: Δ 0.01M; \circ 0.1M; \square 1M; ∇ 4M. Cell wall preparation saturated with calcium as described in the text. — *T. vulgare*; - - - *S. australis*.

Except at very low values of applied suction, it is seen that the drought-resistant *E. calycina* (perennial veldt grass) exhibits the smallest sorptive capacity amongst the four species. Classical experiments have shown that under comparable environmental conditions, there are marked differences in the water requirements of various crop plants and even between varieties of a particular crop, with the amount of water needed for normal growth decreasing from drought-sensitive to drought-tolerant species. It is possible that a common factor enabling perennial veldt grass and other xerophytes to withstand water stress is their smaller water use as compared with more mesophytic species, coupled with the fact that the cell wall water in

xerophytes is held by relatively stronger forces so that it is not easily lost under dehydrating conditions. The latter effect is discussed in relation to the hysteresis phenomenon.

The perennial veldt grass used in this study (an introduced species in Western Australia) is thriving particularly well in its competition with more well-adapted indigenous species (Teoh 1964). From time to time during the last couple of decades, various measures have been tried to eradicate this "weed" from large areas of bushland reserve near Perth, but as yet with little success. Its wiry drought-resistant roots have helped the species to survive recurrent long dry summers with frequent high temperatures.

Salicornia was collected at Pelican Point, W.A., where the roots of these highly salt-tolerant plants are submerged in salt water all the year round. Physiologically, both halophytes and xerophytes have often been regarded as being similar, i.e. both are able to withstand water stress. However, one fundamental difference between the environments in which these plants exist appears to have been overlooked. Although halophytes have been considered to have difficulty in extracting water from their salty habitat, nevertheless their roots generally are bathed continuously in the salt water; in contrast, xerophytes are subject to recurrent long spells of actual water deficiency.

At the very highest relative humidities (approaching free water) it is seen that *Salicornia* has the lowest sorption of the four species. This is probably due to an inherent adaptation of the plant whereby it is able to control the intake of excess water into its system; unless it can do this, it would not thrive in its natural wet habitat. The sorption of electrolyte solutions of various concentrations by *Salicornia* and by wheat over a range of suctions is shown in Figure 2. The values for water potential indicate only that component arising from the applied hydrostatic suction and do not include the lowering of the free energy due to the osmotic component. For all electrolyte concentrations used, sorption by *Salicornia* is not greatly affected by changes in water stress below some 10^3 mb suction. In contrast, wheat increases its solution uptake rapidly at values of water stress below 10^2 mb suction. (Sorption isotherms obtained for the bean and the veldt grass in electrolyte solutions are similar to those of the wheat). It is seen that with increasing salt concentration to 1M, solution uptake by *Salicornia* decreases rapidly but thereafter shows only slight changes. On the other hand, concentrations approaching 1M are necessary to significantly restrict the solution uptake by the wheat, and at 4M a marked reduction is observed, particularly at low water suctions. The characteristic behaviour of *Salicornia* is probably related to its ability to limit the uptake of electrolyte solutions.

Recently the mangroves of Cape York (northern Australia) were studied by Scholander *et al.* (1965) who reported that the xylem sap in these salt-tolerant plants consisted essentially of fresh water. In most of the species they studied, the salt concentration averaged only 1% of that of the sea water. The negative hydrostatic pressure of the sap of *Salicornia* was found to average -50 atm; since the osmotic potential of sea water is about -25 atm, *Salicornia* has an ample margin for extracting fresh water from the ocean.

The ease with which fresh water is available to *S. australis* may be associated with the narrower hysteresis loop as compared with that found for perennial veldt grass (Fig. 1). There are many causes for hysteresis, depending on the nature of the system under study. Hysteresis in a swelling polymer has been interpreted by Cassie (1945) as a reflection of the mechanical constraints present in the resultant swelling gel. Since perennial veldt sorbs the smallest amount of water over most of the range, its cell walls probably have a more tightly bound structure in comparison with wheat, broad bean, and *Salicornia*. It follows that the sorbed water molecules in the wall structure of perennial veldt would probably be held by stronger forces than the majority of those in the other three species. Regardless of how hysteresis is interpreted it is clear that the ability of the cell wall material to retain water when desorbed to a particular water potential varies amongst the four species. The work done in completing an adsorption-desorption cycle, as given by the areas within the respective hysteresis loops, is smallest for the bean and largest for perennial veldt. The cell wall water of drought-sensitive species is likely to be easily depleted under drought conditions. As soil moisture approaches a critical low level, the root probably shrinks slightly due to the withdrawal of water from its AFS to supply the tops. It is possible that permanent damage to the ultrastructure of the cell walls may occur as a result of such volume changes.

The work needed to remove the same quantity of water from various types of cell wall (different matrices with different physicochemical properties) will vary. It seems reasonable that the tenacity with which the cell wall holds onto water would increase as the drought-resisting capacity of the species increases. It is well known that under conditions of water stress the consequent physiological responses in the plant differ from species to species. If a xerophyte and a mesophyte were simultaneously subjected to an identical drying regime, wilting symptoms will appear in the latter long before they do in the former.

Discussing the evidence from various investigations of the relative exchangeability of water in different cell components, Petinov (1962) states that, in general, the ease of exchangeability decreases in the following order: water in cell walls and vascular system, vacuolar water, cytoplasmic water, and water in chloroplasts; this implies that the water in the cell wall and vascular system is relatively free. Under conditions of dehydration the water stored in the microcapillary systems in the fibrillar wall structure can be readily transported and distributed to other cell components where more vital processes are going on.

Adsorption theories applicable to polymeric systems have been reviewed in detail by Chabert and Diemunsch (1962). The applicability of these theories to the present and additional data at low water potentials will be discussed in a separate paper.

IV. CONCLUDING REMARKS

Drought resistance in plant species undoubtedly involves a multiplicity of factors; factors such as sunken stomata, thick cuticle, water-storage mechanisms, etc. obviously contribute to the water economy of plants. However, these specialized adaptations are often characteristic of a particular species or family only. They

assist the plant, in a limited degree, to overcome the initial stages of dehydration. In the final analysis, the drought resistance of a species would depend on its ability to endure increasing intensity of dehydration until internal water deficits become critical. Plants which are adapted to drought conditions and yet possess no obvious morphological features suggesting such adaptability may be called "physiological xerophytes" (Lemée 1946).

The development of an extensive root system is without doubt another means of overcoming inadequate rainfall (e.g. Satoo 1956; see also Palmer, Trickett, and Linacre 1964), but again, not all drought-resistant species possess this characteristic. The roots of perennial veldt grass are generally very fine. In the sandy soils of the Perth area, the adventitious root system of this grass does not penetrate the soil deeper than a depth of about 1 ft. Plants of dry habitats which tap water by means of extensive root systems cannot be regarded as true xerophytes, since they are not subject to actual water stress. More than 50 years ago, Kamerling (1914) had shown that certain so-called xerophytes in fact transpire freely, and he termed them "pseudoxerophytes" in contrast to true xerophytes which have a smaller transpiration rate.

It is suggested that the cell wall plays an important role in the water regime of all arid and semi-arid vegetation with regard to drought resistance, irrespective of whether other adaptive features are developed or not. During periods of drought, xerophytes must possess a greater resistance to the destruction of the integrity of the two-phase nature of the cell wall (solid-liquid system) than do mesophytes. It should be emphasized that it is this greater resistance to desiccation which is considered to be important in this regard rather than the presence of a supplementary water reservoir acting as a buffer in the volumetric sense. It is known that a plant subjected to water stress frequently develops thicker cell walls. "In many plants, the volume of cell wall can reach significant proportions and in xerophytic and sclerophytic tissue the volume of the vacuole can be less than one-half of the total cell volume" (Slatyer and Taylor 1960).

Provided that a rigorously standardized procedure is used in the preparation of cell wall materials, the simple quantitative approach of assessing the comparative drought hardiness of various species as described in this paper may be of practical value in selection trials. However, more studies on plants with varying degrees of known drought resistance would be needed. It is hoped to include the drought-sensitive species *E. brevifolia* and *E. longiflora* (annual veldt grasses) in subsequent studies using this method. Annual veldt grass has a relatively broader leaf lamina and is more mesophytic than perennial veldt grass. A comparison of closely related species having different capacities in withstanding water stress may throw further light on the present findings.

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